

## Supplementary information

# Novel Nicotinamide *N*-methyltransferase Inhibitors

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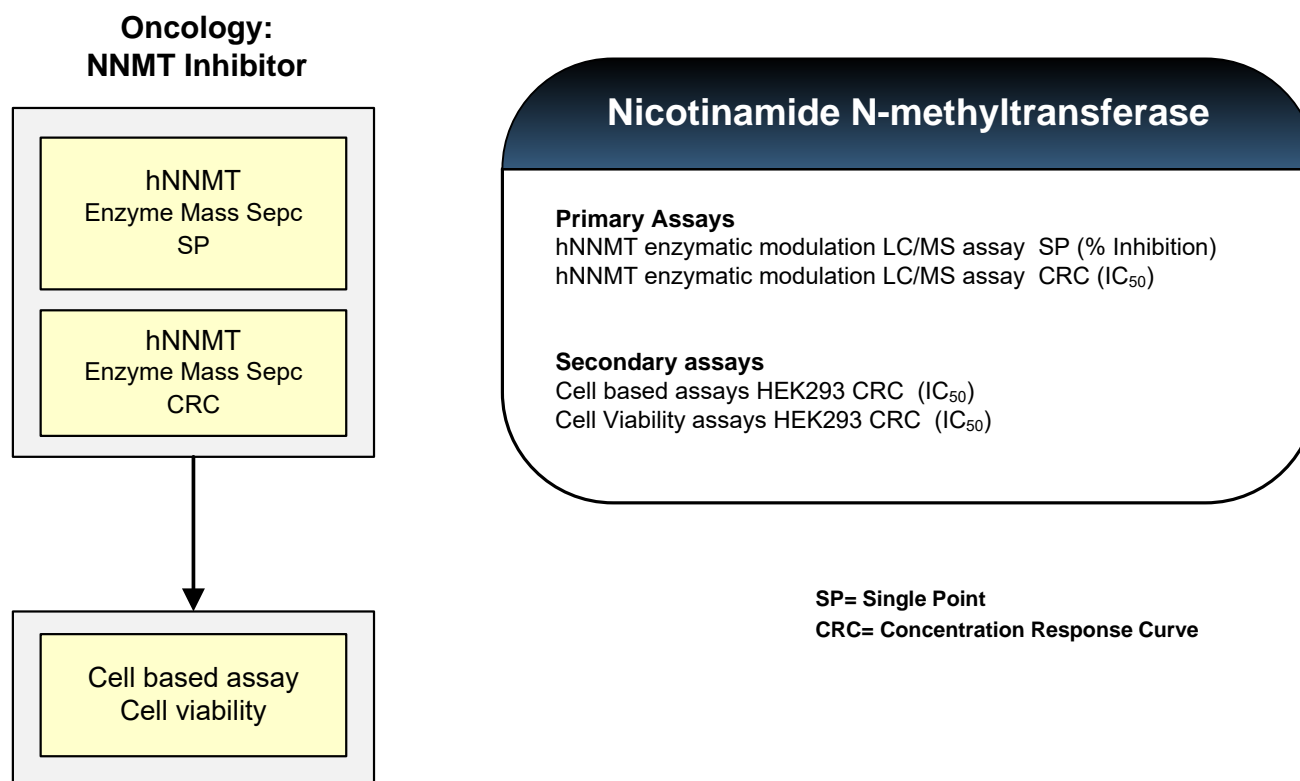
## Oncology – NNMT Inhibitor

Metabolic changes driven by activation of oncogenes, inactivation of tumor suppressors, and pro-tumorigenic mutations are considered a hallmark of cancer. One cancer-associated metabolic enzyme, Nicotinamide N-Methyltransferase (NNMT), catalyses the transfer of methyl groups from the methyl donor S-adenosyl-L-methionine (SAM) to nicotinamide, generating S-Adenosylhomocysteine (SAH) and methylnicotinamide (MNAM). Previous reports have connected the increased expression levels of NNMT in cancer cells with chemotherapy and radiation resistance as well as increased tumor aggressiveness.

Furthermore, downregulation or silencing of NNMT has been shown to increase the sensitivity of carcinoma cells to radiation therapy and decrease tumorigenicity, providing key support for the role of NNMT in promoting treatment resistance and tumorigenesis in cancer cells. Thus, compounds able to modulate the activity of NNMT in the setting of oncology may have therapeutic utility.

- Ulanovskaya OA, Zuhl AM, Cravatt BF. (2013) NNMT promotes epigenetic remodeling in cancer by creating a metabolic methylation sink. *Nature chemical biology*. 9(5):300-6.
- Sauve AA. (2008) NAD<sup>+</sup> and vitamin B3: from metabolism to therapies. *J Pharmacol Exp Ther*. 324(3):883-93.
- van Haren, M.J., Sastre Toraño, J., Sartini, D., Emanuelli, M., Parsons, R.B., \*Martin, N.I. (2016). A rapid and efficient assay for the characterization of substrates and inhibitors of nicotinamide N-methyltransferase. *Biochemistry*, 55(37):5307-15.

## Flow Scheme & Assay Measures

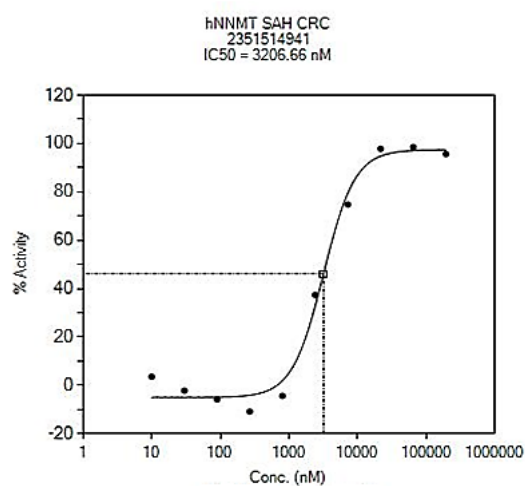


**Figure S1:** Lilly IODD hNNMT Flow Scheme and Assay Measures.

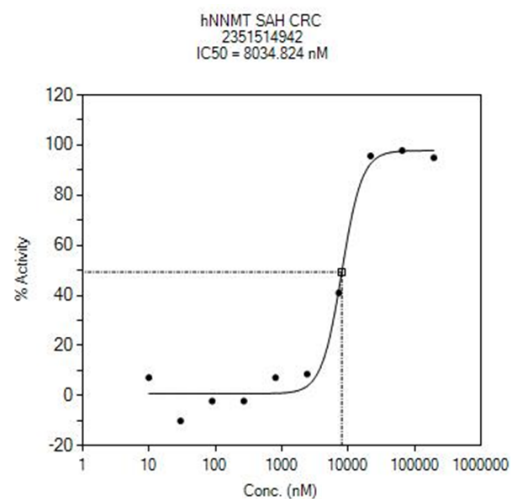
**Table S1.** Inhibitory activity (%) of compounds **4a-n** against human Nicotinamide *N*-methyltransferase (hNNMT) at 10  $\mu$ M.

Compound	hNNMT MNA, % inhibition at 10 $\mu$ M	hNNMT SAH, % inhibition at 10 $\mu$ M
<b>4a</b>	91.29	89.73
<b>4b</b>	40.09	40.16
<b>4c</b>	58.68	56.76
<b>4d</b>	-10.32	-20.17
<b>4e</b>	-5.752	1.149
<b>4f</b>	86.74	87.21
<b>4g</b>	-1.373	5.125
<b>4h</b>	99.34	99.13
<b>4i</b>	-2.608	2.511
<b>4j</b>	8.277	20.07
<b>4k</b>	2.19	ND
<b>4l</b>	-16.81	-11.16
<b>4m</b>	97.99	97.99
<b>4n</b>	-2.382	ND

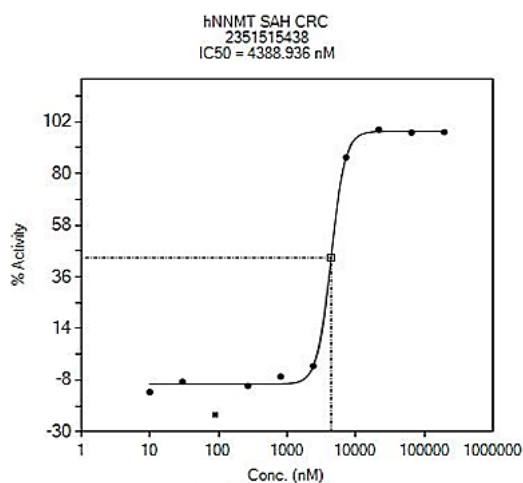
ND: Not determined



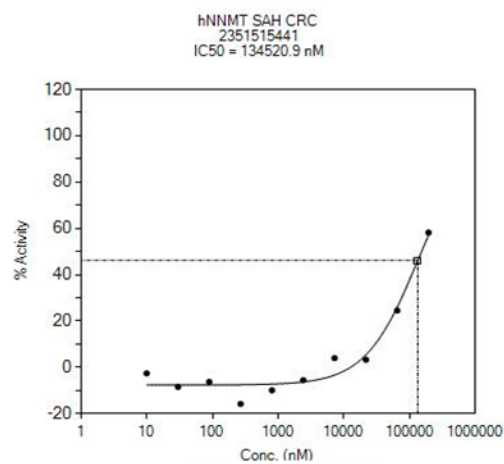
(A)



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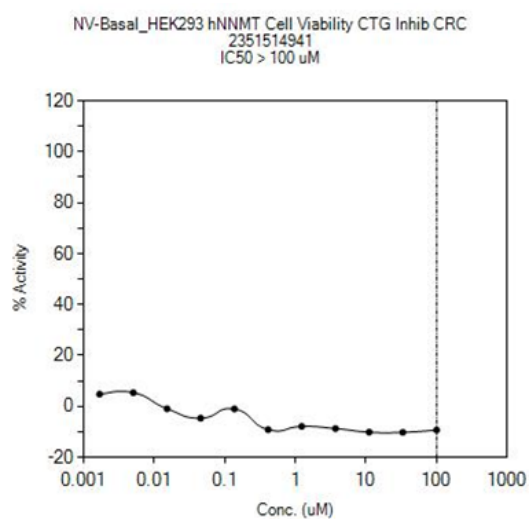


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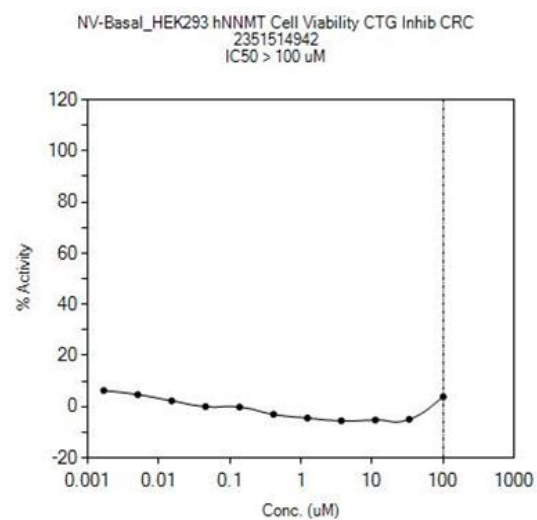


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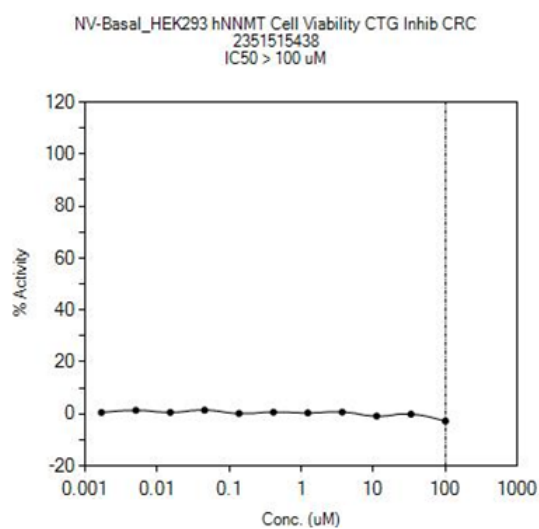
**Figure S2.** Concentration-response curve (CRC, IC<sub>50</sub>) of Nicotinamide *N*-methyltransferase (hNNMT) enzymatic modulation using LC/MS assay via monitoring *S*-adenosyl-L-homocysteine SAH production; for (A) **4a**, (B) **4c**, (C) **4f** and (D) **4h**.



(A)



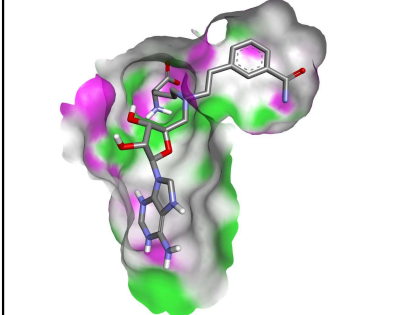
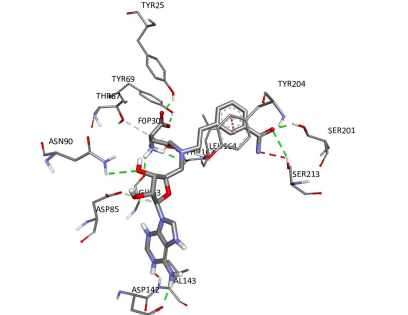
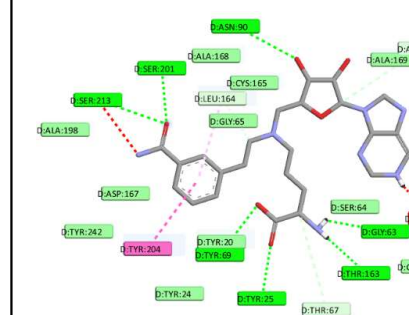
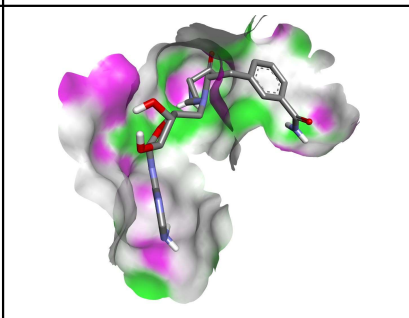
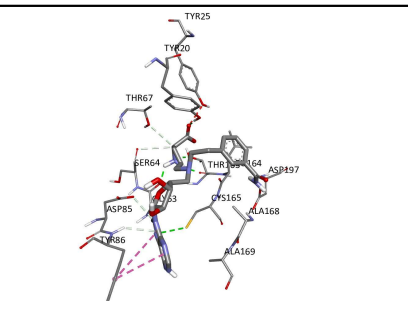
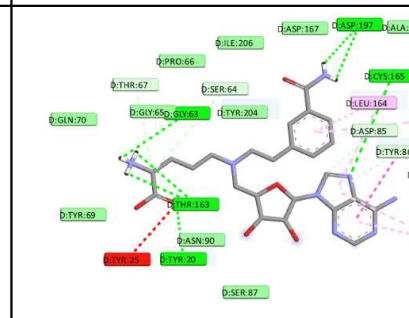
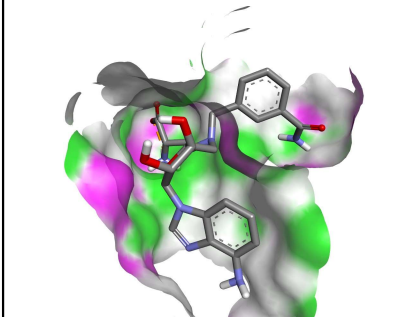
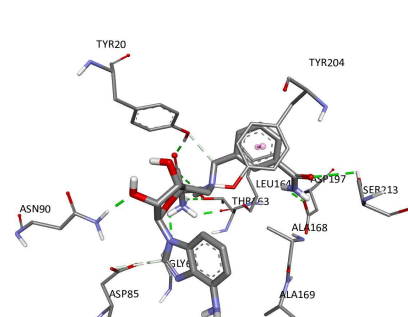
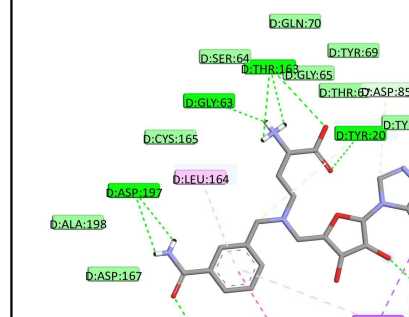
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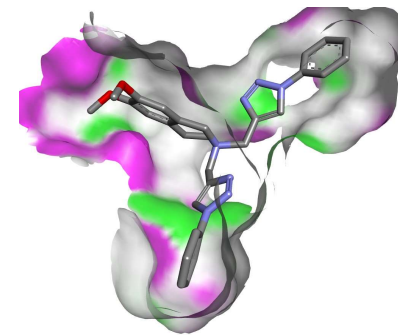
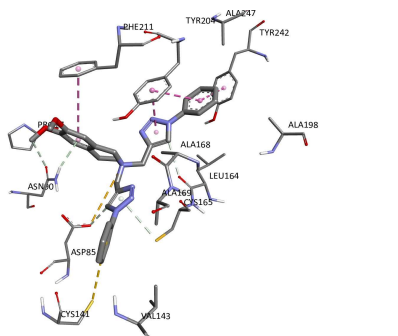
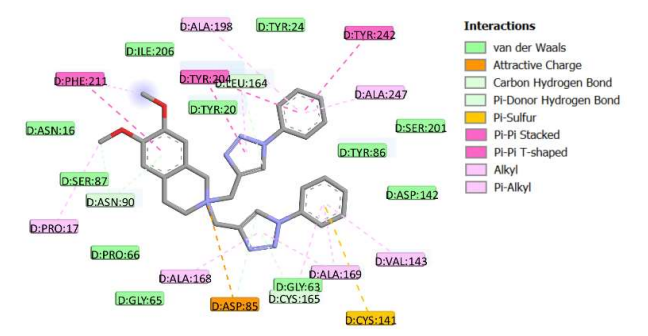
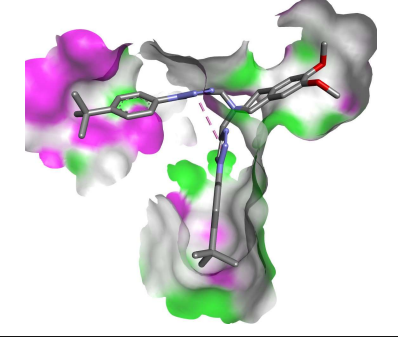
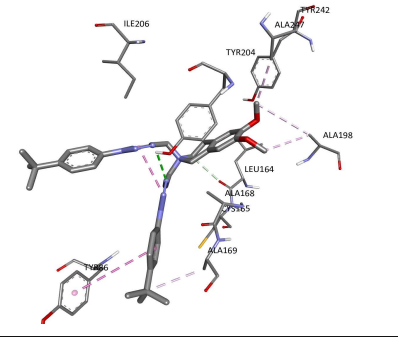
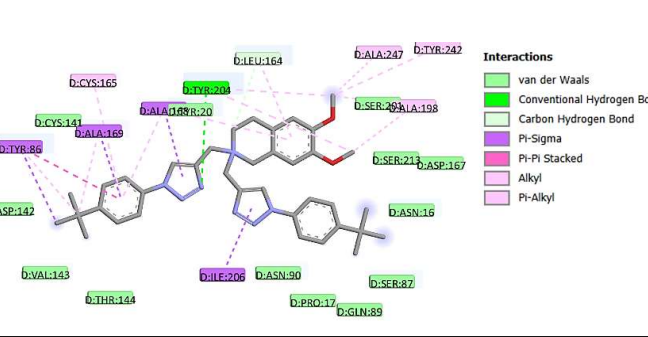
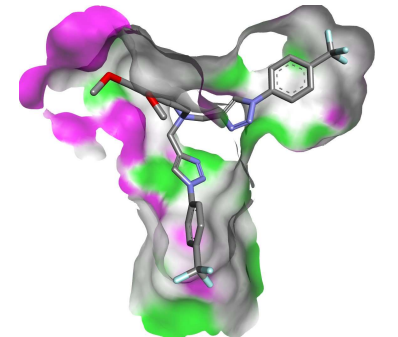
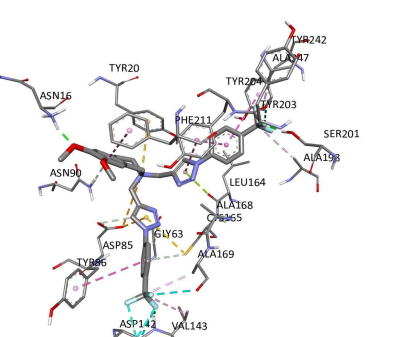
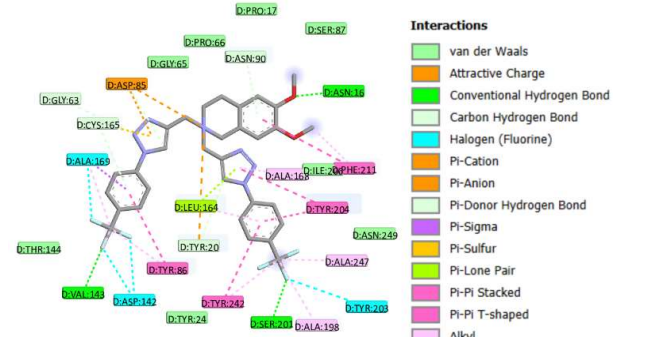
(C)

**Figure S3.** Cell viability assay, the percentage growth inhibition of basal-HEK293 cells for (A) compound **4a**, (B) compound **4c**, and (C) compound **4f**.

**Table S2.** Interactions between hNNMT (6CHH) and **MS2734**, **MvH45** and **4a-n**.

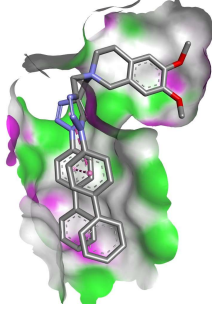
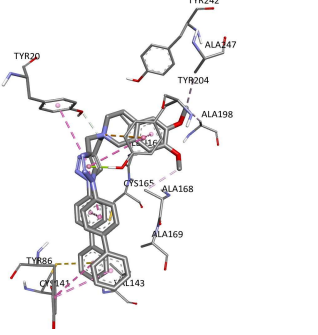
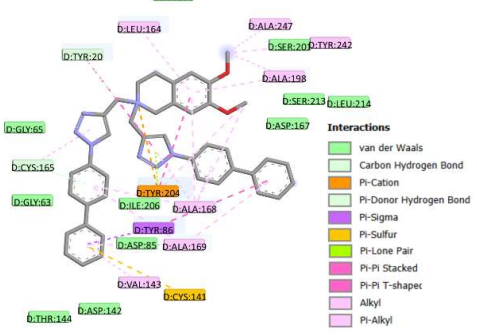
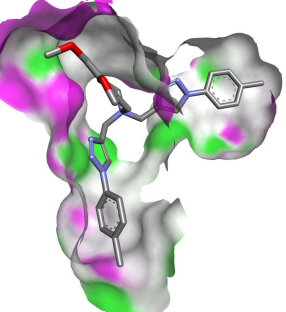
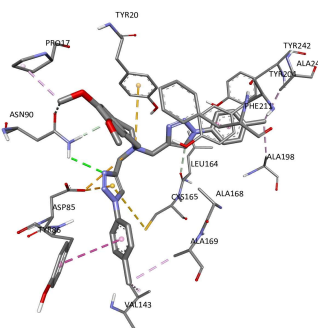
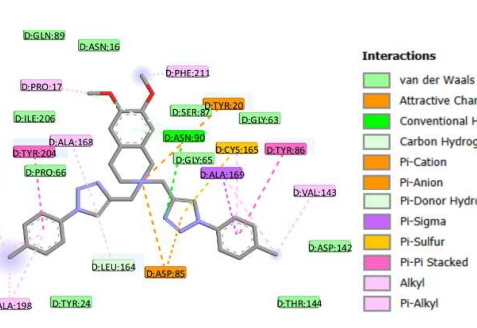
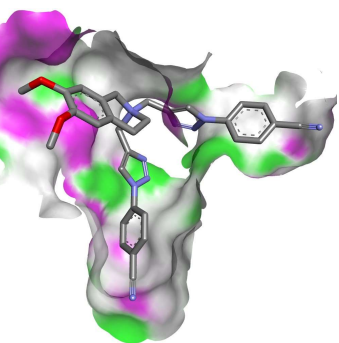
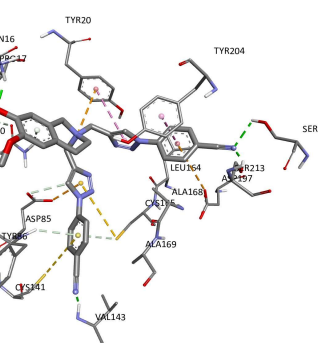
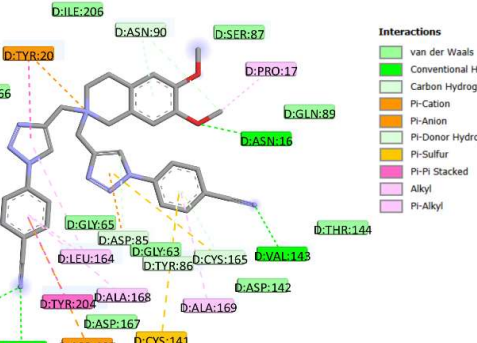
Compound	3D interaction surface	3D interactions	2D interactions
MX2734 in 6CHH			
Re-docked MS2734			
MvH45			

**Table S2.** Interactions between hNNMT (6CHH) and **MS2734**, **MvH45** and **4a-n**.

Compound	3D interaction surface	3D interactions	2D interactions
4a			
4b			
4c			

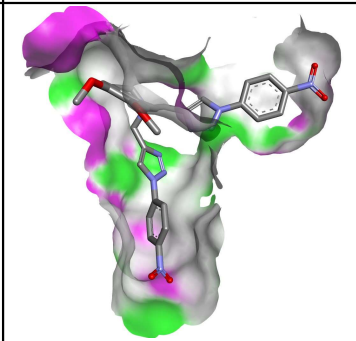
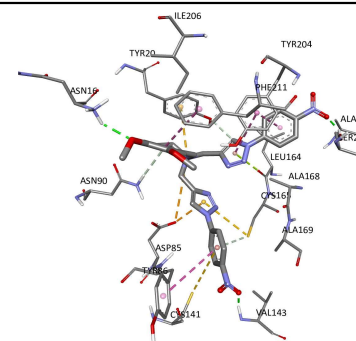
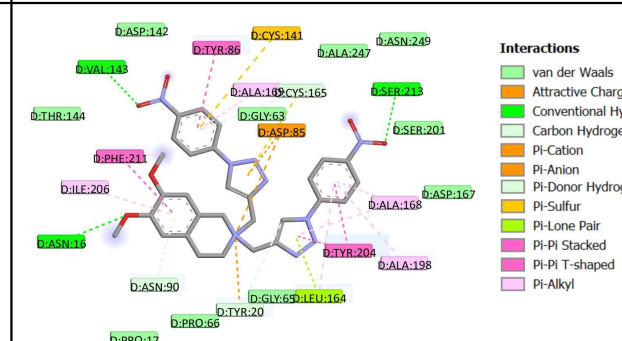
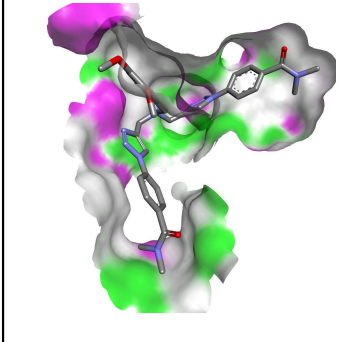
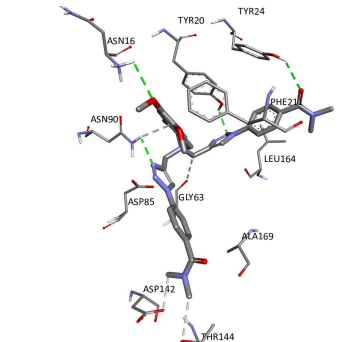
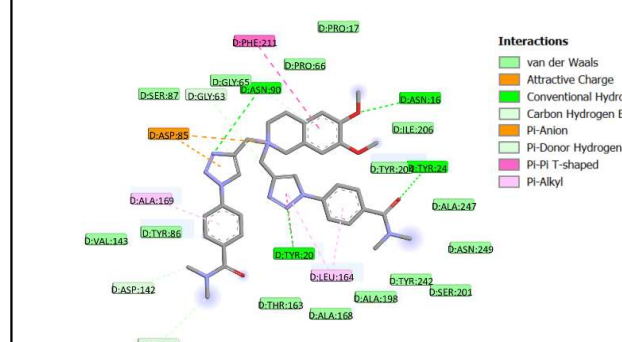
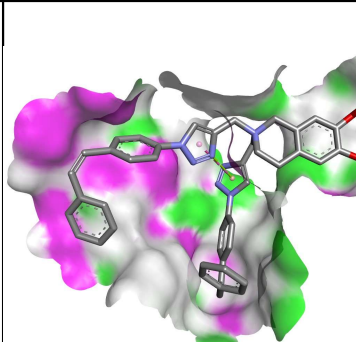
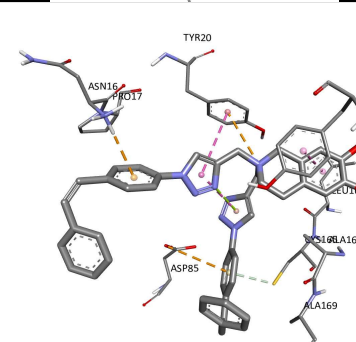
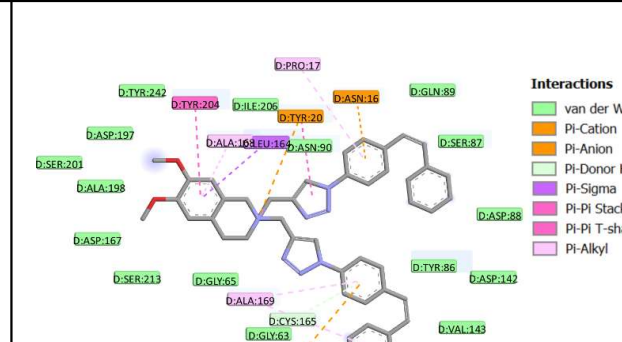


**Table S2 (cont).** Interactions between hNNMT (6CHH) and **MS2734**, **MvH45** and **4a-n**.

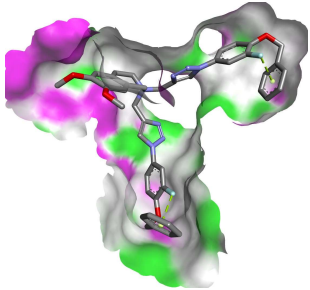
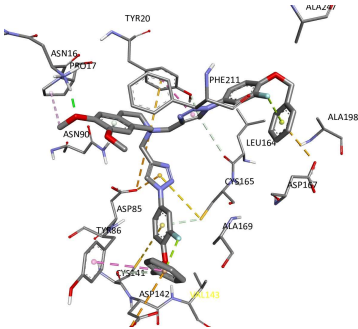
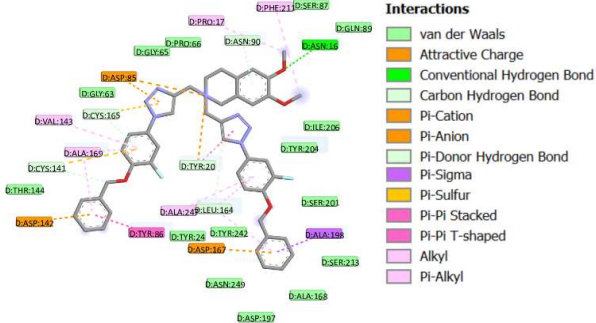
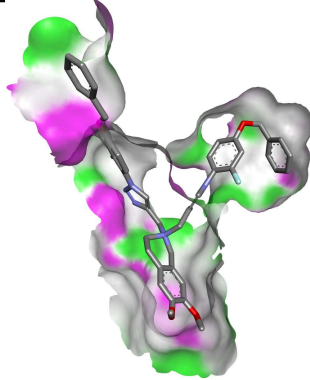
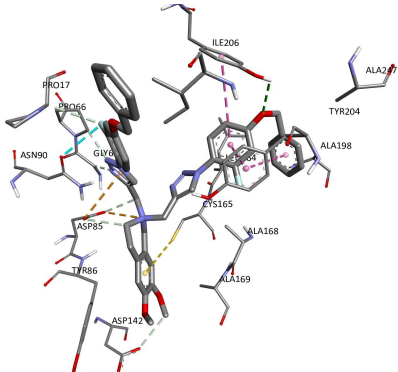
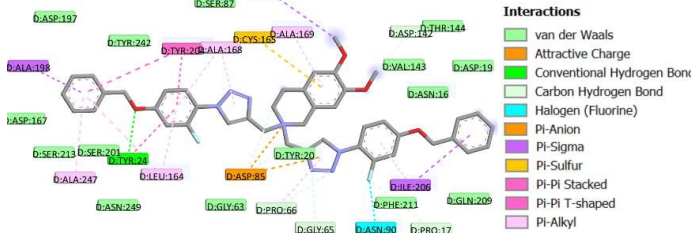
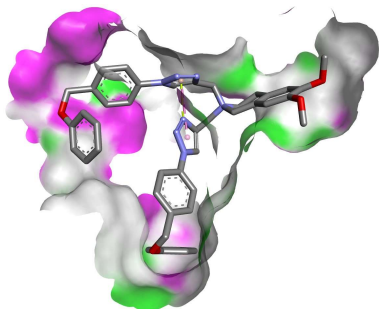
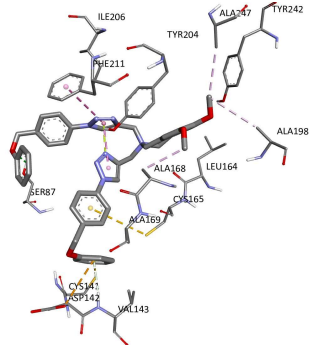
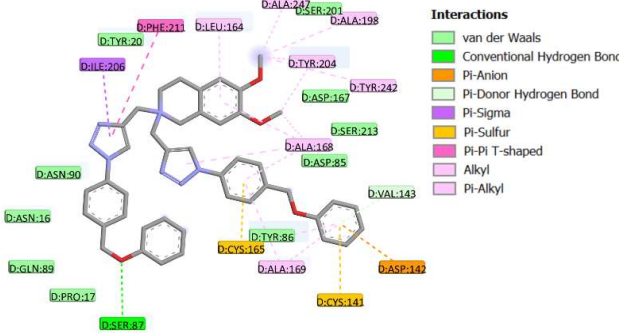
Compound	3D interaction surface	3D interactions	2D interactions
4d			
4e			
4f			



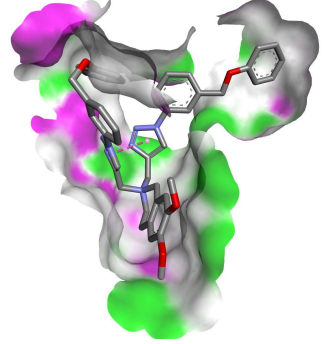
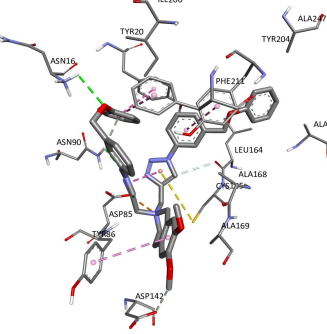
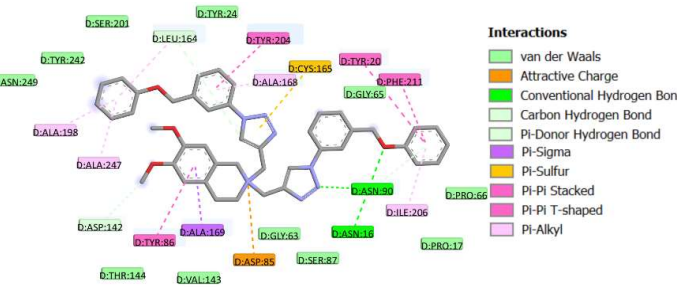
**Table S2 (cont).** Interactions between hNNMT (6CHH) and **MS2734**, **MvH45** and **4a-n**.

Compound	3D interaction surface	3D interactions	2D interactions
4g			
4h			
4i			

**Table S2 (con.).** Interactions between hNNMT (6CHH) and **MS2734**, **MvH45** and **4a-n**.

Compound	3D interaction surface	3D interactions	2D interactions
4j			
4k			
4l			

**Table S2 (cont.).** Interactions between hNNMT (6CHH) and **MS2734**, **MvH45** and **4a-n**.

Compound	3D interaction surface	3D interactions	2D interactions
4m			
4n	